EXPRESS MAIL NO. EV719383907US

DECLARATION OF IAN MACLACHLAN, PH.D. UNDER 37 CFR 1.132

I, Ian MacLachlan, Ph.D., declare as follows:

1. All statements herein made of my own knowledge are true, and statements made on information or belief are believed to be true and correct.

2. I currently hold the position of Chief Scientific Officer for Protiva Biotherapeutics, Inc, located in Burnaby, British Columbia, Canada, a licensee of U.S. Patent Application Serial No. 09/431,594, entitled "Lipid-Nucleic Acid Particles Prepared Via a Hydrophobic Lipid-Nucleic Acid Complex Intermediate and Use For Gene Transfer." My field of expertise is nucleic acid delivery and molecular gene therapy. I have authored over twenty-five publications in the field of nucleic acid delivery technology, molecular gene therapy and molecular genetics, and I am a member of the American Society of Gene Therapy and the Oligonucleotide Therapeutics Society. A copy of my *Curriculum Vitae* is attached hereto (Exhibit A).

3. I have read and am familiar with the above-identified patent application, and the Office Action mailed March 7, 2006 by the United States Patent & Trademark Office in the above-referenced patent application. It is my understanding that U.S. Patent No. 5,820,873 (Choi *et al.*) and U.S. Patent No. 5,885,613 (Holland *et al.*) are cited by the Examiner as allegedly disclosing nucleic acid particles that meet the structural limitations of the particles claims in the above-referenced patent application.

4. I submit this Declaration for the purpose of providing additional evidence regarding the ability of the lipid hydration-extrusion method described in Choi *et al.* Holland *et al.* to be used to encapsulate nucleic acids. Specifically, this declaration is provided to supplement my prior declarations submitted December 21, 2005 and May 30, 2005, which demonstrated that the methods of Choi *et al.* and Holland *et al.* could not be used to efficiently encapsulate plasmid DNA, and to present additional data regarding the

ability of the methods of Choi *et al.* and Holland *et al.* to encapsulate oligonucleotides as opposed to plasmids.

5. The following experiments were performed under my supervision. A lipid solution comprising DOPE:DODAC:PEG-ceramide C14 (82.5:7.5:10 molar percent), was prepared by dissolving these lipids in chloroform. This lipid solution contained a total of 2.22 μmoles lipid. Nitrogen gas was then used to drive off chloroform to form a lipid film. The lipid film was then hydrated at 37°C with 2 ml nuclease-free water containing 50 or 100 μg of plasmid DNA or a 20mer oligodeoxynucleotide (ODN) to generate liposomal samples with nucleic acid to lipid ratios of 22.5 or 45 μg/μmol. The resulting suspension was subjected to 5 rounds of freezing in liquid nitrogen and thawing in a 37°C water bath, to increase homogeneity of the resulting multilamellar vesicles. These samples were then extruded 10 times through 2 stacked 100 nm polycarbonate filters using a 10-mL Extruder (Northern Lipids Inc.) and nitrogen gas at 300-600 psi. Particle size analysis was performed on these samples before and after extrusion using a Malvern Instruments Zetasizer. Nucleic acid encapsulation was determined using the membrane impermeable probes Picogreen and Ribogreen that fluoresce in the presence of plasmid DNA and ODN, respectively. The proportion of nucleic acid entrapped with liposomes was determined by measuring the fluorescence intensity of these probes before and after the addition of the detergent Triton X-100. The results of these experiments are provided in Exhibit B, attached hereto.

6. It is clear from these results that the widely used lipid hydration-extrusion method described by Choi *et al.* and Holland *et al.* is not suitable for preparing liposomes encapsulating plasmid DNA. Plasmid encapsulation was very poor at the two drug:lipid ratios (15%) examined, and, more importantly, at least 98% of the plasmid DNA was lost on the extrusion filters. In contrast, liposomes of the same lipid composition could be prepared by this lipid hydration-extrusion method that encapsulated ODN. Nucleic acid entrapment was 49% prior to extrusion and 38% in the 120 nm particles following 5 rounds of extrusion. Additionally, there was only modest ODN loss from extrusion, with 79% of the initial ODN retained in the extruded samples. Of the total input ODN, 31%

was entrapped in the extruded samples as compared to 0.2% for the plasmid formulations prepared at the same nucleic acid:lipid ratio (22.5 μg/μmol).

7. In view of the foregoing, it is my opinion that neither Choi *et al.* nor Holland *et al.* teach or suggest the presently claimed nucleic acid-lipid particles wherein the nucleic acid is plasmid DNA, because neither Choi *et al.* nor Holland *et al.* teach or suggest: (1) nucleic acid-lipid particles wherein plasmid DNA is encapsulated in the liposome and is resistant in aqueous solution to degradation with a nuclease; or (2) methods for making such liposomes. Moreover, I believe that it has been unequivocally demonstrated that the lipid hydration extrusion methods described in Choi *et al.* and Holland *et al.* do not lead to the presently claimed nucleic acid lipid particles wherein plasmid DNA is encapsulated in the lipid and is resistant in aqueous solution to degradation with a nuclease.

8. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Ian MacLachlan, Ph.D.

Date Sept. 7, 2006.

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Exhibit A

CURRICULUM VITAE
IAN MACLACHLAN, PH.D.

BIOGRAPHIC DATA

Name:	Ian MacLachlan
Address:	1544 Grant Street, Vancouver, BC, Canada, V5L 2Y2
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EDUCATION

May 1988 - Jun 1994	**Ph.D. (Biochemistry)** University of Alberta, Edmonton, Canada, & Department of Molecular Genetics, University of Vienna, Austria.
Sep 1985 - May 1988	**B.Sc. (Biochemistry)** University of Alberta, Edmonton, Canada.
Sep 1982 - May 1984	**Biological Sciences** University of Calgary, Calgary, Canada.

EXPERIENCE

Sep 2000 - Present	**Chief Scientific Officer** Protiva Biotherapeutics, Inc., Burnaby, BC, Canada.	Development of Non-Viral Nucleic Acid Delivery Systems for Cancer, Inflammatory and Infectious Disease.
Jul 1996 - Aug 2000	**Team Leader / Research Scientist** Inex Pharmaceuticals Corporation Burnaby, BC, Canada.	Non-Viral Cancer Gene Therapy. Suicide Gene Therapy, Pharmacology, Vector Development, Tumor Modeling, Inducible Gene Expression.
Jul 1994 - Jun 1996	**Research Fellow** Howard Hughes Medical Institute Department of Internal Medicine University of Michigan, USA. Supervisor: Dr. G.J. Nabel	TNF Mediated Activation of NF-κB and the HIV LTR Adenoviral Gene Therapy for Restenosis. The Role of NF-κB in Vertebrate Development.
May 1988 - Jun 1994	**Graduate Student** Lipid and Lipoprotein Research Group University of Alberta, AB, Canada. & Dept. of Molecular Genetics University of Vienna, Austria. Supervisor: Dr. Wolfgang Schneider	Molecular Genetics of the Lipoprotein Receptor Family. Characterization of the Receptor Mediated Uptake of Riboflavin Binding Protein Including Cloning and Characterization of the *rd* Mutant.
Jan 1988 - Apr 1988	**Undergraduate Research** University of Alberta, AB, Canada. Supervisor: Dr. Wayne Anderson	Computerized Sequence Analysis of Lipoproteins, Crystallography of Membrane Proteins.

Sep 1987 - Dec 1987	**Undergraduate Research** University of Alberta, AB, Canada. Supervisor: Dr. Wolfgang Schneider	Purification and Characterization of Apolipoprotein VLDL-II, an Inhibitor of Lipoprotein Lipase.
Summer 1987	**Undergraduate Research** Bamfield Marine Station, Canada. Supervisor: Dr. Ron Ydenberg	Behavioral Analysis of the Polychaete, *Eudystilia vancouveri.*
May 1983 - Dec 1986	**Programmer** Canadian Hunter Exploration Ltd. Calgary, Alberta, AB, Canada.	Programming of Oil and Gas Reservoir Simulations and Data Analysis Tools Used to Guide the Exploration Efforts of an Oil and Gas Company.

TRAINING

June 2004	American Society of Gene Therapy/ USFDA	Long Term Follow-up of Participants in Human Gene Transfer Research
March 2003	American Society of Gene Therapy / USFDA	Non-Clinical Toxicology in Support of Licensure of Gene Therapies
Sept 2002	Protiva Biotherapeutics	WHMIS and Chemical Safety Retraining
Sept 2002	TLM Consulting	Basic GMP Training
June 2002	American Society of Gene Therapy / USFDA	Clinical Gene Transfer Comprehensive Review Course
Apr 2002	TLM Consulting	Introduction to Gene Therapy Clinical Trials and GLP/GMP
Jul 2001	Protiva Biotherapeutics	Cytotoxic Drug Training
May 2001	American Society of Gene Therapy / USFDA	Clinical Gene Transfer Training Course
Jun – Sep 1998	Leadership Edge Consulting	Lab-to-Leader Training Program Project Management, Coaching, Team Management
Oct 1997	Pape Management Consulting	Project Management Training II
May 1997	University of British Columbia	Radionuclide Safety and Methodology
Feb 1997	Pape Management Consulting	Project Management Training I

AWARDS AND DISTINCTIONS

1995 - 1998	Medical Research Council of Canada Fellowship
1993	Mary Louise Imrie Graduate Award, Faculty of Graduate Studies and Research, University of Alberta
1992 - 1994	Austrian Fonds zur Förderung der Wissenschaftlichen Forschung (Austrian Ministry of Science Scholarship)
1989 - 1993	Heart and Stroke Foundation of Canada Research Trainee
1982	Rutherford Scholarship

AFFILIATIONS AND MEMBERSHIPS

1999 - 2002	Science Council of British Columbia - Health Technology Committee
1998 - Present	American Society of Gene Therapy, Member
2004 - Present	American Society of Gene Therapy - Non-viral Vectors Committee

PUBLICATIONS

Judge, A.D., Sood, V., Shaw, J.R., Fang, D., McClintock, K. and MacLachlan, I., *Synthetic siRNA Stimulate the Mammalian Innate Immune Response in a Sequence Dependent Manner*, In Press: Nature Biotech, 2005.

Heyes, J., Palmer, L.R., Bremner, K. and MacLachlan, I., *Cationic Lipid Saturation Influences Intracellular Delivery of Encapsulated Nucleic Acids*, In Press: Journal of Controlled Release, 2005.

Ambegia, E.G., Ansell, S., Cullis, P.R., Heyes, J.A., Palmer, L.R. and MacLachlan, I., *Stabilized Plasmid-Lipid Particles Containing PEG-Diacylglycerols Exhibit Extended Circulation Lifetimes And Tumor Selective Gene Expression*, In Press: Biochim Biophys Acta, 2005.

Jeffs, L.B., Palmer, L.R., Ambegia, E.G., Giesbrecht, C., Ewanick, S. and MacLachlan, I., *A Scalable, Extrusion Free Method for Efficient Liposomal Encapsulation of Plasmid DNA,* In Press: Pharmaceutical Research, 2005.

MacLachlan, I. and Cullis, P.R., Diffusible-PEG-Lipid Stabilized Plasmid Lipid Particles, In Press: In: Non-viral Vectors for Gene Therapy, Huang, L., Hung, M.C. and Wagner, E., Eds. Academic Press, 2005.

Finn, J., MacLachlan, I., Cullis, P.R., *Factors Limiting Autogene-based Cytoplasmic Expression Systems,* In Press: FASEB Journal, 2005.

Finn, J., Lee, A., MacLachlan, I., Cullis, P.R., *An Enhanced Autogene-based Dual Promoter Cytoplasmic Expression System Yields Increased Gene Expression*, Gene Ther. 2004 Feb;11(3):276-83.

Palmer, L.R., Chen, T., Lam, A.M.I., Fenske, D.B., Wong, K.F., MacLachlan, I., Cullis, P.R., *Transfection Properties of Stabilized Plasmid-Lipid Particles Containing Cationic PEG Lipids,* Biochim Biophys Acta.1611:204-16, 2003.

Cullis, P.R., MacLachlan, I., Fenske, D.B., *Lipid Based Systems for Systemic Gene Therapy*, Journal of Liposome Research, In Press.

Fenske, D.B., MacLachlan, I., Cullis, P.R., *Stabilized Plasmid-Lipid Particles: a Systemic Gene Therapy Vector*, Methods in Enzymology, 346: 36-71, Academic Press, San Diego, 2002.

Pampinella, F., Lecheardeur, D., Zanetti, E., MacLachlan, I., Benhaouga, M., Lukacs, G.L., Vitiello, L., *Analysis of Differential Lipofection Efficiency in Primary vs Established Myoblasts*, Molecular Therapy, 5:161-169, 2002.

Fenske, D.B., MacLachlan, I., Cullis, P.R., *Long-circulating Vectors for the Systemic Delivery of Genes*, Current Opinion in Molecular Therapeutics, 3 (2):153-158, 2001.

Pampinella, F., Pozzobon, M., Zanetti, E., Gamba, P.G., MacLachlan, I., Cantini, M., Vitiello, L., *Gene Transfer In Skeletal Muscle by Systemic Injection of DODAC Lipopolyplexes*, Neurological Science, 21:S971-973, 2000.

MacLachlan, I., Cullis, P.R., Graham, R.W., Synthetic Virus Systems for Systemic Gene Therapy. In: *Gene Therapy: Therapeutic Mechanisms and Strategies*, Smyth-Templeton, N., Lasic, D.D., (Eds.) Marcel Dekker, New York, 2000.

MacLachlan, I., Cullis, P.R., Graham, R.G., *Progress Towards a Synthetic Virus for Systemic Gene Therapy*, Current Opinion in Molecular Therapeutics, 1: 252-249, 1999.

Mortimer, I., Tam, P., MacLachlan, I., Graham, R.W., Saravolac, E.G., Joshi, P.B., *Cationic Lipid Mediated Transfection of Cells in Culture Requires Mitotic Activity*, Gene Therapy, 6: 403-411, 1999.

Wheeler, J.J., Palmer, L., Ossanlou, M., MacLachlan, I., Graham, R.W., Hope, M.J., Scherrer, P., Cullis, P.R., *Stabilized Plasmid Lipid Particles: Construction and Characterization*, Gene Therapy, 6: 271-281, 1999.

Wu, B., Woffendin, C., MacLachlan, I., Nabel, G.J., *Distinct Domains of IκB-κ Inhibit Human Immunodeficiency Virus Type I Replication Through NF-κB and Rev*, J. Virology, 71(4):3161-3167, 1997.

MacLachlan, I., Steyrer, E., Hermetter, A., Nimpf, J., Schneider, W. J., *Molecular Characterization of Quail Apolipoprotein II: Disulphide-bond Mediated Dimerization is Not Essential For Inhibition of Lipoprotein Lipase*. Biochem. J. 317: 599-604, 1996.

Elkin, R.G., MacLachlan, I., Hermann, M., Schneider, W.J., *Characterization of the Japanese Quail Oocyte Receptor for Very Low Density Lipoprotein and Vitellogenin*, J. Nutrition, 125: 1258 - 1266, 1995.

MacLachlan, I., Nimpf, J., Schneider, W. J., *Japanese Quail Apo-VLDL-II: cDNA Sequence and Comparison to Chicken Apo-VLDL-II, a Specific Inhibitor of Lipoprotein Lipase*. Atherosclerosis: 109: 62,1994.

MacLachlan, I., Schneider, W.J., *Avian Riboflavin Binding Protein Binds to Lipoprotein Receptors in Association With Vitellogenin*. J. Biol. Chem., 269: 24127-24132, 1994.

MacLachlan, I., Nimpf, J., White, H.B., Schneider, W.J., *Riboflavinuria in the rd Chicken: 5' -Splice Site Mutation in the Gene for Riboflavin Binding Protein*, J. Biol. Chem. 268 : 23222-23226, 1993.

MacLachlan, I., Nimpf, J., Schneider, W.J., *A Point Mutation in the Gene for Riboflavin Binding Protein Leads to Activation of Alternate Splicing Pathways Causing Riboflavinuria in the rd Chicken*. Fed. Amer. Soc. Exper. Biol. Jour., 7: 1091, 1993.

Schneider, W.J., Vieira, A.V., MacLachlan, I., Nimpf, J., Lipoprotein Receptor Mediated Oocyte Growth. In: *Cellular Metabolism of the Arterial Wall and Central Nervous System; Selected Aspects*; Schettler, G., Greten, H. ,Habenicht , A.J.R. (Eds.) Springer-Verlag, Berlin, 1993.

SELECTED ABSTRACTS

MacLachlan, I. *Plasmid Encapsulation and Tumor Gene Expression of Stable Lipid Particles, a Systemic Gene Therapy Vector*, Lipids and Biomembranes: New Technologies, October 2-5, 2002.

MacLachlan, I., Ambegia, E., McClintock, K, Jeffs, L., Palmer, L., Meitz, A., &. Cullis, P.R, *Disease Site Targeting and Tumor Specific Gene Expression of Stable Plasmid-Lipid Particles for Systemic Gene Delivery*, Eleventh International Conference on Cancer Gene Therapy, July 11-12, 2002.

Finn, J.D., Lee, A., MacLachlan, I., Cullis, P.R. *The Development and Characterization of a Cytoplasmic Expression System Based on the T7 Phage RNA Polymerase Protein*. American Society of Gene Therapy, 5th Annual Meeting, June 5-9, 2002.

Sandhu, A., Verheul, R., de Jong, S., MacLachlan, I., Cullis, P. *Enhancing the Intracellular Delivery Characteristics of Stable Plasmid-Lipid Particles*. American Society of Gene Therapy, 5th Annual Meeting, June 5-9, 2002.

MacLachlan, I., Ambegia, E., Meitz, A., Tam, P., Cullis, P.R. *Programmable pharmacokinetics, disease site targeting and tumor specific gene expression of stable plasmid-lipid particles for systemic gene delivery*. Tenth International Conference on Gene Therapy of Cancer, Dec 13-15, 2001

Kyla, C., Cullis, P., Carr, K., Murray, M., Shaw, J., Palmer, L., MacLachlan, I. *Effect of Cationic Lipid Structure on the Pharmacology and Resulting Transfection Activity of Stabilized Plasmid Lipid Particles (SPLP).* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Wong, T., Wong, K., Cullis, P., Fenske, D., MacLachlan, I., Sandu, A., Lo, E. *Optimizing the Transfection Potency of Stable Plasmid-Lipid Particles Based on the Endosomal Release Parameter.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Cullis, P., Finn, J., MacLachlan, I. *The Development and Comparison of Three Cytoplasmic Expression Systems Based on the T7, T3 and SP6 Phage RNA Polymerase Proteins.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Ansell, S., Currie, K., Ambegia, E., Cullis, P., Carr, K., MacLachlan, I., Murray, M. *Stabilized Plasmid Lipid Particles Containing Diacylglycerol Anchored PEG Lipids:In vitro and In Vivo Characterization.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Ambegia, E., Cullis, P., Fenske, D., Palmer, L., MacLachlan, Ian., Murray, M. *Programmable Pharmacokinetics, Disease Site Targeting and Tissue Specific Gene Expression of Stable Plasmid-Lipid Particles for Systemic Gene Delivery and Expression.* American Society of Gene Therapy, 4th Annual Meeting, May 30-June 3 2001.

Finn, J., MacLachlan, I., Cullis, P. *The development and comparison of three cytoplasmic expression systems based on the T7, T3 and SP6 phage RNA polymerase proteins.* Gene Therapy 2001: A Gene Odyssey, January 6-11, 2001.

MacLachlan, I., Fenske, D., Ambegia, E., Murray, M., Cullis ,P. *Programmable Pharmacokinetics, Disease Site Targeting and Tissue Specific Gene Expression of Stable Plasmid-Lipid Particles for Systemic Gene Delivery and Expression,* Gene Therapy 2001: A Gene Odyssey, January 6-11, 2001.

MacLachlan, I., Fenske, D., Palmer, L., Wong, K., Lam, A., Chen, T., Cullis, P. *Elimination of PEG-Lipid mediated Inhibition of Transfection.* Third Annual Meeting of the American Society of Gene Therapy, May 31-June 4, 2000.

Ahkong, L., Airess, R., Harasym, T. Hope, M., Klimuk, S., Leng, E., MacLachlan, I., Semple, S.C., Tam, P. and Cullis, P.R., *Pre-clinical Studies with Liposomal Mitoxantrone: Formulation, Pharmacokinetics, Toxicity and Efficacy,* 7th Liposome Research Days, April 12-15, 2000.

MacLachlan, I., Tam, P., Lee, D., Thompson, J., Geisbrecht, C., Lee, A., Thomson, V. and Cullis, P.R., *A Gene Specific Increase In the Survival of Tumor Bearing Mice Following Systemic Non-viral Gene Therapy,* 7th Liposome Research Days, April 12-15, 2000.

MacLachlan, I., Palmer, L.R., Fenske, D.B., Lam, A.M.I., Wong, K.F., Chen, T., Cullis, P.R. *A Flexible Platform for Enhancing the Transfection Potential of PEG-Lipid Containing Transfection Reagents.* Gene Therapy: The Next Millennium, January 6-12 2000.

Graham, R.W., Tam, P., Lee, D., Thompson, J., Giesbrecht, C., Lee, A., Thompson, V., MacLachlan, I. *A Gene Specific Increase in the Survival of Tumor Bearing Mice Following Systemic Non-Viral Gene Therapy.* American Society of Gene Therapy, 2nd Annual Meeting, June 9-13, 1999.

MacLachlan, I.,Tam, P., Ayres, S., Buchkowsky, S., Lee, A., Saravolac, E.G.,Graham, R.W. *Encapsulated Non-Viral Gene Delivery Systems for Suicide Gene Therapy.* American Society of Gene Therapy, 1st Annual Meeting, May 28-31st, 1998.

Buchkowsky, S., Ayres, S., Graham, R., MacLachlan, I. *Liposomal Encapsulation of Ganciclovir Results in Improved Pharmacokinetics and Biodistribution.* American Society of Gene Therapy, 1st Annual Meeting, May 28-31st, 1998.

Exhibit B

Sample	Nucleic Acid Entrapment Efficiency (%)		Total Nucleic Acid in Extruded Sample vs Before Extrusion (%)	Input Nucleic Acid Entrapped & Recovered after Extrusion (%)	Particle Size (nm) with SD	Polydispersity with SD
	Before Extrusion	After Extrusion				
Empty vesicles	n/a	n/a	n/a	n/a	179 ± 7	0.19 ± 0.02
Plasmid DNA 22.5 µg/µmol	32 ± 6	15 ± 10	1.4 ± 0.5	0.2 ± 0.2	140 ± 13	0.23 ± 0.05
Plasmid DNA 45 µg/µmol	12 ± 5	7*	2.0*	0.1*	116*	0.24*
20mer ODN 22.5 µg/µmol	49 ± 1	38 ± 3	79 ± 8	31 ± 6	120 ± 6	0.29 ± 0.05

All values n = 3 samples ± SD
*Single sample (very difficult to extrude)
Note: Prior to extrusion, all samples possessed very large mean vesicle sizes (i.e., > 10,000 nm)